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Variation in wheat (*Triticum aestivum*) advance lines and released cultivars for traits associated with nitrogen use efficiency under N limiting environment

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ABSTRACT

A subset of 19 genotypes was formulated on the basis of mean values for various traits related to nitrogen use efficiency from 175 genotypes comprising advance breeding lines and released Indian wheat (*Triticum aestivum* L.) cultivars observed under hydroponic condition. This subset was further evaluated in the pipe under nitrogen limiting environment for the various traits related to nitrogen use efficiency and other agronomic parameters. Variability study shows wide ranges of variation for the traits under studies. Nitrogen uptake efficiency (NUpE) shows more variation in comparisons to nitrogen utilization efficiency (NUtE) under N limiting condition. All of below ground and above ground traits except plant height shows positive correlation with nitrogen use efficiency (NUE). A positive correlation between shoot dry weight and root dry weight indicates no conflict for resource allocation and both can be simultaneously improved through selection in the breeding programme to improve NUE. Cluster analysis showed that cluster III found to be best performing for NUE trait and can be used in selection or to generate desirable genetic variability. In this study, the derivative of the cross HD 2967/DT 2761 show high root dry weight (RDW), shoot dry weight (SDW) and thus finally lead to high NUE under limiting N and can be used further in breeding programmes.

Key words: Association, Cluster, NUE, NUpE, NUtE, Root traits,Variation, Wheat

Wheat was one of the first domesticated food crops and for 8000 years has been the basic staple food for major civilizations in Europe, South Asia, and North Africa. Wheat is grown on more than 240 million ha and continues to be the most leading important food grain source for humans (Lumpkin 2009). Green revolution in India was mainly based on wheat and it has taken the production level of wheat in India from 6.46 million tonnes from an area of 9.75 million ha in 1950-51 to more than 97 million tonnes from an area of about 30.71 million ha with productivity 3.2 tonnes/ha during 2016-17 (IIWBR 2016-17). Wheat pivotal for food security, stabilizing the yield through improved varieties and better production system is much essential against the wide fluctuation in yield particularly, the northwestern plain zone of India (Yadav *et al*. 2017). Wheat is an important source of both carbohydrates and protein not only in human but also livestock nutrition (Shewry 2009). Increased nitrogen (N) fertilization, irrigation and use of high yielding dwarf varieties were important factor in the enhancement of wheat production achieved during the 20th century (Yadav *et al*. 2010). Globally, fertilizer nitrogen (N) application has increased rapidly over the past several decades, from 32 million tonnes in 1970 to around 100 million tonnes in 2010 and it is expected to increase to 130–150 million tonnes/year by 2050 (Matson *et al.* 1998). However, there are problems associated with N fertilizer use as leaching of N can cause eutrophication of water (Vitousek *et al*. 1997), and increases emissions and of the greenhouse gas nitrous oxide (N_2O) from agricultural soils (Malyan *et al*. 2016). Due to increasing human health concerns and pressure in meeting stringent consumer standards, agricultural practices are shifting towards the development of suitable nitrogen (N) fertilizers (Khalil *et al*. 2009), practices and crop genotypes which result in high N utilization efficiency, and can sustain crop production. Economic reasons beside environment and health concern thus make it necessary for farmers to adopt management systems that improve NUE.

NUE is a term used to indicate the grain production per unit of available nitrogen in the soil (Moll *et al*. 1982). Globally, cereal grain NUE is estimated at about 33%, with 42% and 29% in developed and developing countries, respectively (Raun and Johnson 1999). The utilization of N by higher plants involves several processes including,

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uptake mechanisms, storage, translocation, reduction and incorporation into organic forms (Moll *et al*. 1982). NUpE and NUtE are the two primary components of N use efficiency (Moll *et al*.1982). Since the breeders in the developing world including India were preoccupied with yield improvement and stabilization, exploitation of genetic variation for improving NUE genetically ignored. Under both N sufficient and N limiting environment search for plant genotypes with greater N use efficiencies, either in a strict physiological sense [increased carbon (C) gain/N], or in an agronomic sense (increased dry matter or protein yield per unit plant N or per unit N applied/available to the crop (Andrews *et al*. 2004). There is hardly any information regarding the extent of genetic variability for NUE and its component traits largely because of difficulty in phenotyping. Wheat plants largely absorb the available N through roots and therefore root traits like root weight and root length are highly relevant for better acquisition but has been largely ignored again because of difficulty in scoring.

Screening of wheat under different N environment gives contrasting results for NUE because of differential response of the genotype. According to Le Gouis *et al*. (2000), variation in NUE under limiting N supply was largely because of variation in uptake rather than utilization. Since the most of selection for wheat improvement are being carried out under N rich environment, role of root traits in better N acquisition has been overlooked. Both NUE and root traits are complex in nature, depending on both genetic and environmental factors along with their interactions (Li *et al*. 2015). The present investigation was therefore planned to study the role of root traits and other important yield component traits for NUE in N limiting environment. Besides this knowledge about the clustering, direct and indirect contribution of different traits to NUE would be highly useful for formulating selection criteria and can be used to choose the parents without making crosses before the initiation of hybridization programme.

MATERIALS AND METHODS

A set of 175 wheat genotypes were grown under the hydroponic system for 8 weeks in a temperature and photoperiod controlled condition maintained at National Phytotron Facility at IARI, New Delhi, India under two different environments, i.e. with high N and N limiting solution in three replications during *rabi* 2014-15. The experimental material was grown under 25/22 °C day/night temperature with the light intensity of 300 μmol/m^2/s using cool fluorescent lamps in 10/14 hr of dark and light timing using an automatic timer with 65–70 % relative humidity (Ayalew *et al*. 2014). The data were recorded for rooting depth (RL), root dry weight (RDW), shoot dry weight (SDW) and shoot nitrogen (N) % for each individual plant. The rooting depth (cm) was measured at harvest by the graduated ruler from the shoot base to longest root tip. After removing root and shoot, both were dried in an oven at 60ºC for 4 days before measuring their weight. Shoot N% was estimated by Micro- Kjeldahl method. A subset of 19 genotypes from 175 genotypes was formulated on the basis of mean values for different traits. Extreme seven genotypes for N content in shoot (gN) from N rich environment, i.e. N responsive genotypes, extreme six for gN from N limiting, i.e. N efficient genotypes and extremes six for root dry weight under N rich were selected for further study.

This subset of 19 genotypes as in was used for the association study for the traits influencing NUE and the extent of variability for different traits in the soil filled to PVC pipe. The PVC pipes of 100 cm length and 23 cm diameter were mounted in open field of experimental farm, Division of Genetics, IARI, New Delhi (28˚35 N, 77˚12 E and 228.16 m asl) in two replication during *rabi* 2015-16 as shown in Sandy loam soil used to fill the pipe was first solarized under sun and then sieved. N content in the soil was analyzed in the sample taken randomly. The soil was then weighed and mix properly with P_2O_5 @ 100 kg/ha, K @ 60 kg/ha and 30 kg/ha N. Surface sterilized uniform two seeds of each genotype were sown in individual PVC pipe. Plants were raised up to flowering stage and then harvested carefully with intact root by removing the soil with water pressure. The data for plant height (cm), rooting length (cm), number of tillers/plant, number of spikes/plant, spike length (cm), shoot dry weight (g), root dry weight (g) and N content for each genotypes were generated. Shoot and root dry weights were taken after drying shoot and root in the oven at 60ºC for 4 days. N content was estimated by the Kjeldahl method.

Nitrogen use efficiency (NUE) and its component traits were calculated as per Moll *et al*. (1982).

Nitrogen use efficiency (NUE) = NUpE × NUtE

$$\text{Nitrogen uptake efficiency (NUpE)} = \frac{\text{Total N in plant}}{\text{N supply (g/plant)}}$$

where, total N in plant = N % in shoot × shoot dry weight (gm) = g N in shoot; N supply (g/plant) = Total N present in N limited soil= 0.286 g (from calculation).

$$\text{Nitrogen utilization efficiency (NUtE)} = \frac{\text{Shoot dry weight (g)}}{\text{Total N in plant}}$$

Analysis of variance (ANOVA) was carried out as per complete randomized design with OPSTAT software (Sheoran *et al*. 1998). Mean value of two replicas for each trait was used for Karl Pearson correlation coefficient and path analysis. Karl Pearson correlation coefficient and path analysis were carried out with the software R-studio (https://www.rstudio.com/). Cluster analysis was carried out by using Euclidean distance with Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to create cluster by DARwin 6.0.17 (http://darwin.cirad.fr/).

RESULTS AND DISCUSSION

Genetic variability for the characters

The analysis of variance revealed statistically significant differences among the genotypes for all the characters under

Table 1 Mean of characters under study for 19 genotypes in pot culture under N limiting

Characters	Mean ± SE	Range
Root length (RL-cm)	74.737 ± 1.402	55.00-97.00
Root dry weight (RDW-gm)	2.378 ± 0.442	1.03-4.05
Shoot dry weight (SDW-gm)	6.663 ± 0.477	3.92-8.16
Plant height (PH-cm)	66.816 ± 0.981	46.00-83.50
Numbers of tillers/plant (TN)	4.553 ± 0.425	3.00-6.50
Numbers of spike/plant (SN)	3.474 ± 0.405	1.75-5.00
Spike length (SL-cm)	11.782 ± 0.292	10.00-13.75
Nitrogen (N%)	1.119 ± 0.183	0.60-1.44
Gram nitrogen in shoot (gN)	0.075 ± 0.072	0.03-0.10
Nitrogen uptake efficiency (NUpE)	0.261 ± 0.135	0.126-0.355
Nitrogen utilization efficiency (NUtE)	92.771 ± 2.232	69.31-166.03
Nitrogen use efficiency (NUE)	23.298 ± 0.891	13.70-28.53

study. More than 100% of the variation was observed for most of the traits except root length, plant height and spike length (Table 1). The range for root length among the studied genotypes was from 55- 97 cm and for root dry weight it was 1.03 - 4.05 g.

The range for shoot dry weight was 3.92 - 8.16 g whereas for gram nitrogen in shoot (gN), it was 0.03 - 0.10 gm. Similarly for NUpE it was 0.126 - 0.355, for NUtE, it was 69.31 - 166.03 and finally for NUE the range was found to be 13.7 - 28.53. This shows that sufficient variability existed for NUE and component traits under N limiting environment. Presence of genetic variation is a prerequisite for making the genetic gain for the trait through selection. The continuous breeding effort has narrowed down the available genetic variability in the elite gene pool of most of the cereal crops; however, germplasm and related species harbor enough variability for NUE traits. Genetic variation for NUE parameters in modern cereals have been reported in several studies as Ortiz-Monasterio *et al*. (1997), Le Gouis *et al*. (2000), Bingham *et al*. (2011), Gaju *et al*. (2011) and Guttieri *et al*. (2016). Our study confirm the finding of Ortiz-Monasterio *et al*. (1997), Le Gouis *et al*. (2000), Bingham *et al*. (2011) that variability for NUpE is more than NUtE under N limiting environment , however, Gaju *et al*. (2011) reported more importance of NUt under N limiting environment of wheat. Higher variability present in NUpE might be due to high variation present in the traits that influence NUpE like root morphology (root length, root density, root surface area and average root diameter), root membrane N transporter system or/and also interaction with soil microbes present at rhizosphere. These traits influence N acquisition in plant and finally NUpE too. NUtE on the other hand is decided through assimilation, translocation, and remobilization of N from root to leaves and finally leaves to grain. Any setback in these processes may influence NUE adversely.

The relationship between characters under study in pot culture

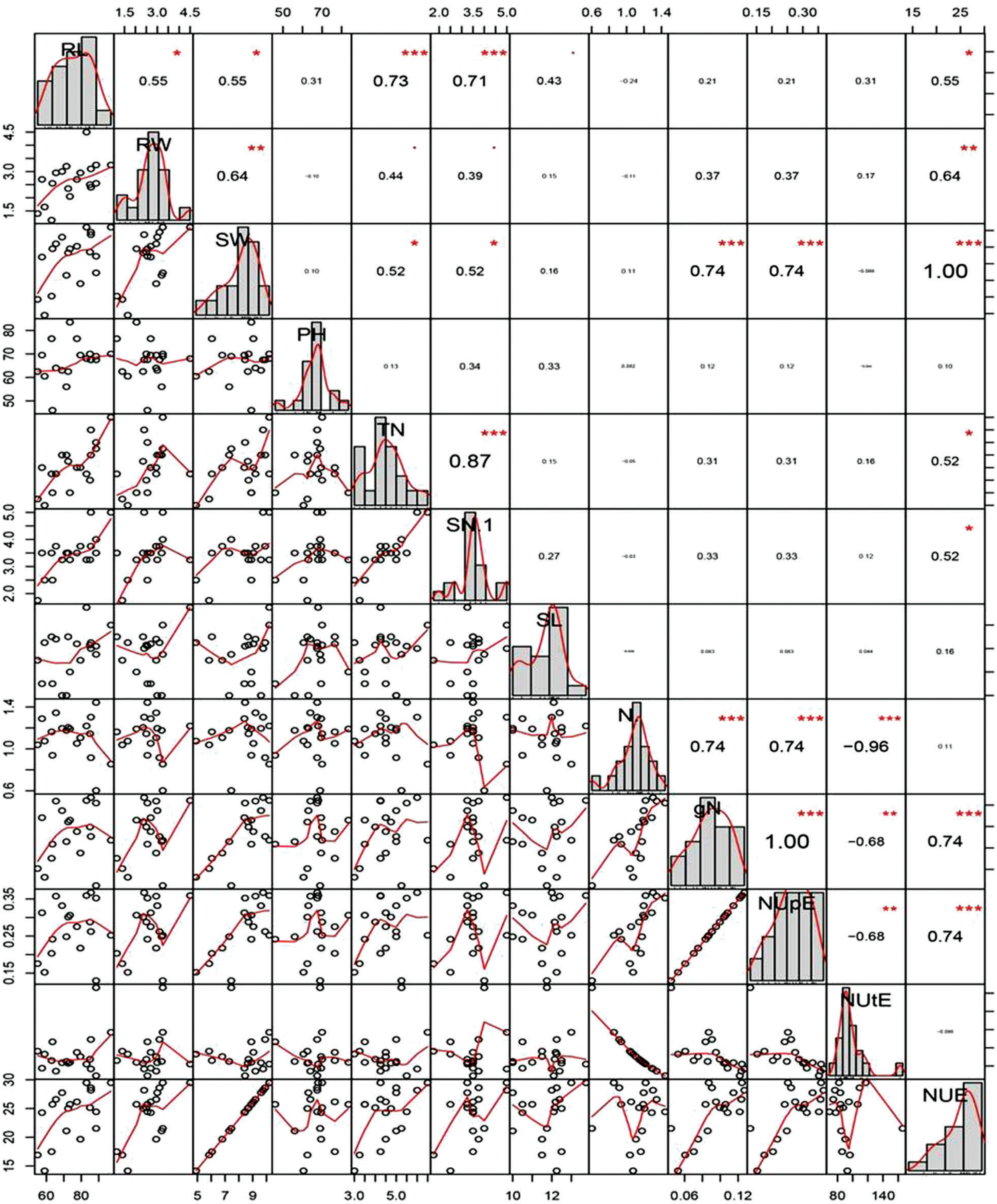
Knowledge about the relationship between different component traits with NUE can help in the formulation of selection strategies for further improvement. Correlation worked out among the different traits under study is presented in (Fig 1). NUE had a significant and strong correlation with component traits like root length, root dry weight, shoot dry weight, numbers of tillers/plant, numbers of spikes per plant, N content in the shoot (gN) and NUpE. SDW, direct function to NUE is positively and significantly associated with root length, root dry weight, numbers of tillers/plant, number of spikes/plant and NUpE. No negative correlation between root traits and above ground yield component traits except height provide opportunities to the breeders for further yield consolidation by selecting largely unexplored root traits. The best possible scenario for improving NUE is through simultaneous improvement in NUpE and NUtE, however significant negative correlation between these traits limits the scope for such improvement. Due to difficulty in large-scale phenotyping of root traits in breeding programmes, only limited genotypes were used for phenotyping of root traits under pot culture for association studies. Association of root trait and nutrient uptake was similarly studied in rice (Ju *et al*. 2017), wheat (Narayanan *et al*. 2014) and maize (Lynch 2007) and found that selecting root length, lateral root dispersion, root biomass and density also improve the water and nutrient uptake. As around half of N available in the soil is made available to plants through mass flow, prolific root system is more desirable for better accessibility to nutrient; however, increase in root length density (root length per unit of soil volume) beyond critical threshold (1 cm/cm^3) is useless for N uptake (van Noordwijk 1983).

To further understand the response of NUE, it becomes imperative to partition such association into direct and indirect effects of component character through path analysis. Our study (Table 2) clearly showed that only trait which shows strong direct effect on NUE under N limiting environment of wheat was NUpE which in fact largely comes through better N acquisition mainly through root traits. RDW being correlated with RL contributed both directly or indirectly towards NUE through each other.

Table 2 Direct (diagonal) and indirect (off diagonal) effects of NUE under low N

	RL	RDW	TN	SN	NUpE	NUE
RL	0.238	0.153	-0.005	0.046	0.122	0.554
RDW	0.131	**0.278**	-0.003	0.025	0.213	0.645
TN	0.173	0.123	**-0.007**	0.057	0.177	0.522
SN	0.169	0.109	-0.006	**0.065**	0.186	0.522
NUpE	0.051	0.104	-0.002	0.021	**0.571**	0.745

Residual= 0.23305



*** Significant at 0.1%, ** Significant at 1 % and * significant at 5%

RL= Root length, RW= Root Dry weight, PH= Plant height, TN= Tillers number, SN1= no. of spikes, SL= Spike length, N= Nitrogen%, NUpE= Nitrogen uptake efficiency, NUtE= Nitrogen utilization efficiency and NUE= Nitrogen use efficiency

Fig 1 Correlation between the traits under study.

Grouping of genotypes

On the basis of mean Euclidean and complete linkage method, 19 subset genotypes grouped into three clusters. Among the clusters, maximum genotypes (13) were included in cluster III followed by Cluster II (4) and cluster I accommodated 2 genotypes respectively (Fig 2). Cluster mean for various traits (Table 3) revealed that considerable differences were noticed between the cluster means for different characters. Cluster III was showing maximum mean value for most of the characters under

Table 3 Cluster mean of different traits of subset

Cluster	RL	RW	PH	TN	SN	SL	N%	NUpE	NUtE	NUE	Score	Rank
I	73.75 (2)	2.20 (2)	64.75 (3)	4.12 (3)	3.25 (2)	12 (1)	0.83 (3)	0.13 (3)	129.5 (1)	17.27 (3)	23	III
II	69.12 (3)	1.96 (3)	65.37 (2)	4.25 (2)	3.25 (2)	11.9 (2)	1.08 (2)	0.24 (2)	92.65 (2)	22.83 (2)	22	II
III	76.61 (1)	2.53 (1)	67.57 (1)	4.71 (1)	3.5 (1)	11.7 (3)	1.17 (1)	0.28 (1)	87.14 (3)	24.36 (1)	14	I

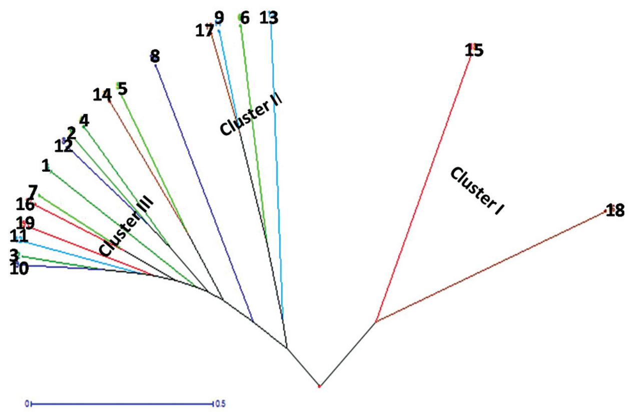


Fig 2 Tree diagram based on UPGMA illustrated the genetic relationship among genotypes.

study largely because of non accommodation of poor scorer for any of the trait in the group. Cluster II have mediocre genotypes thus rank II and genotype no. 15 and 18 were in separate branch but can accommodated in same cluster, i.e cluster I which rank III that recorded 5 characters in negative direction (3 score). Therefore, use of genotypes in cluster III would be desirable to generate the variability in the desired direction. It is suggested that hybridization among the genotypes of above said clusters would produce segregants for more than 1 economic character. The ranking of genotypes on the basis of mean value for high NUE under N limiting environment suggest, genotypes No. 10, 16, 19 ranked 1-3 and genotype no. 15 ranked last which too concede with tree diagram. High NUE of theses genotypes are due to high shoot weight and high root weight. Interestingly there seems to be no conflict between roots and shoot traits for resource allocation and genotypes with high root weight also have high shoot weight and can be integrated for higher yield realization through improved sink capacity.

It can be concluded from our study that under the condition of narrowing down of exploitable genetic variation for above ground traits, sufficiently large variation present in root traits and their positive association with yield component traits and NUE can be safely selected for further yield improvement with minimum N foot print. Root weight probably combining high root area, root length, root volume, number of root tips and being easily measurable under condition like hydroponic can be safely explored for simultaneous improvement of NUE and yield. Thus by combining the higher source (root weight) and high sink (shoot weight) capacity, these can be evolved into more N efficient genotypes for exploitation under N limiting environment. The derivative of the cross HD 2967/DT 2761 were showing maximum value for soil exploring root traits, i.e. RDW, SDW and thus finally lead to high NUE under limiting N and therefore can be used further in breeding programmes. Cluster III seemed to be most promising for NUE traits thus, selection and use of these genotypes be useful to generate desirable genetic variability.

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